Analysis of Cigarette Smoke Fraction by Combined Gas Chromatography—Infrared Spectrophotometry

SIR: Recently, an instrument was described (4, 7) which is claimed to eliminate the need for manual transfer of collected gas chromatographic peaks to infrared absorption cells. The instrument permits the shunting of an eluting peak directly into a gas absorption cell and provides a rapid (45 seconds) scanning of the infrared spectrum of the sample from 2.5 to 15.0 microns. The utility of the equipment was demonstrated experimentally by separation and spectral analysis of components in simple synthetic mixtures of compounds (7). As with many instruments, such separations do not prove the value of the equipment when used in studies on complex mixtures such as natural products. To determine such value, we have used the instrument to study components in an ether codistillate of cigarette smoke condensate. Details of the performance of the equipment and on the possible identities of some components in the codistillate are presented herein.

EXPERIMENTAL

Sample Preparation. Smoke condensate (980 grams) was partitioned between 4 liters of ether and 4 liters of $1N$ aqueous NaOH. The alkaline layer was washed with an additional 4 liters of ether, and all ether solutions were combined. Bases were removed from the combined ether solutions with two successive extractions (3.6 liters each of $0.2N$ HCl). The resulting ether solution of neutrals was washed free of mineral acid with small portions of water, dried over Na_2SO_4, and the ether slowly distilled. The distillate, which gradually assumed a light yellow color during collection, was then concentrated on Stedman and spinning band columns to a volume of 5 ml. as previously described (6).

Gas Chromatography. Usually, 100-μl. aliquots of the concentrated distillate were sufficient for both gas chromatography and infrared spectral analysis of individual peaks. The gas chromatographic portion of the instrument was a Loenco Model 70 (no endorsement implied) equipped with a thermal conductivity detector and fitted with dual columns (10 feet $\times$ 0.25-inch o.d.) packed with 20% Apiezon L on Chromosorb W. Operating conditions were as follows: column temperature, 55° C. for 20 minutes, then programmed at 2° per minute to 100° C.; flow rate, 60 ml. per minute of helium; injector temperature, 200° C.; detector temperature, 250° C.

Infrared Spectral Analysis. Using the Wilks chromatograph - spectrograph, selected gas chromatographic peaks were shunted into the infrared absorption cell heated to 200° C. Between successive samples the cel was flushed with carrier gas by opening the entrance and exit valves. Infrared spectra of authentic compounds were obtained in the same manner as the unknowns in the mixture.

RESULTS AND DISCUSSION

The gas chromatogram of the ether codistillate and tentative identifications of some components are included in Figure 1. Peaks were identified by infrared spectral characteristics and gas chromatographic retention times. The infrared spectra of some peaks indicated the presence of more than one component, as would be expected in a complex mixture of this type.

Although the infrared spectra were somewhat lacking in resolution, they were of sufficient value when used in conjunction with retention data to provide further evidence of peak identities. For example, the spectra of peaks 4 and 5 were indicative of simple aldehydes. A study of the elution pattern of a homologous series of authentic normal aldehydes showed that the peaks in question elute between n-butyraldehyde and n-valeraldehyde. Therefore, peaks 4 and 5 appeared to be branched chain aldehydes and probably C_5 compounds.

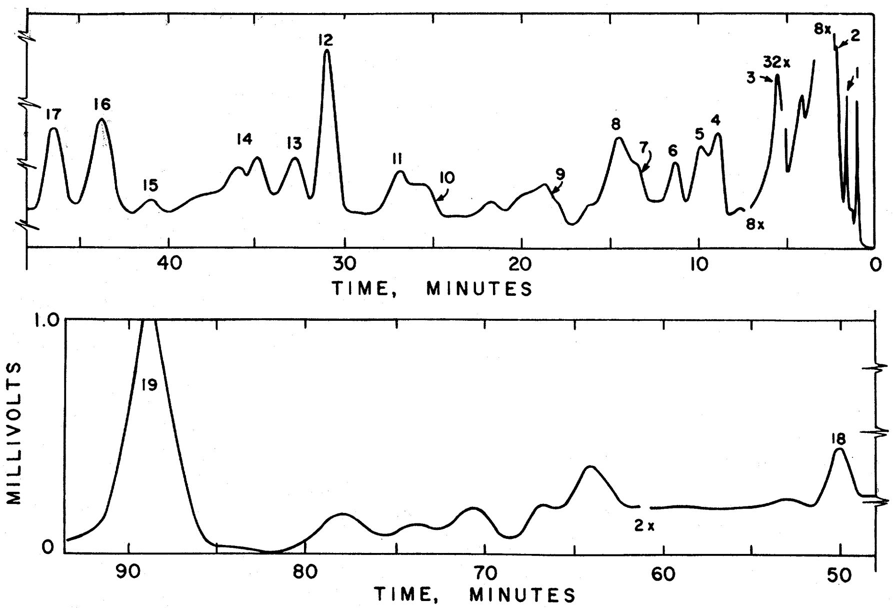

Figure 1. Chromatogram of ether codistillate of cigarette smoke condensate

1. Acetaldehyde
3. Ethyl acetate
4. Isovaleraldehyde
5. α-Methylbutyraldehyde
6. n-Propyl methyl ketone
12. Toluene
16. Ethylbenzene and others
17. m-Xylene
18. Styrene, o-xylene, and others
19. Dipentene

Additional studies with authentic compounds showed that peaks 4 and 5 corresponded to isovaleraldehyde and α-methylbutyraldehyde. The spectrum corresponding to peak 6 resembled that of methyl ethyl ketone, a known smoke component. However, the retention time of authentic methyl ethyl ketone did not match the unknown and further studies with authentic ketones showed that peak 6 was identical to *n*-propyl methyl ketone in retention time and infrared spectral characteristics. The spectrum of peak 6 was distinctly different from that of diethyl ketone which had a similar retention time.

The spectrum of peak 18 (Figure 2) resembled that of styrene, another known smoke component, except for a strong band just below 6 microns and a weak band just below 14 microns. Also, the retention time of authentic styrene was identical with that of peak 18. However, *o*-xylene eluted in the same time and the weak absorption just below 14 microns could be attributed to a relatively small amount of this component. The presence of carbonyl absorption in peak 18 indicated the presence of at least one other component.

The retention time and infrared spectrum (Figure 3) of the major peak in the chromatogram (peak 19) were identical with those of dipentene, a common constituent of tobacco smoke.

These examples illustrate the utility of this instrument for separating complex mixtures of natural products. Even with only partial chromatographic resolution—e.g., peaks 4 and 5 in Figure 1—useful spectra can be obtained for some components. Compounds with a wide range of boiling points can be determined easily (peak 1, acetaldehyde, b.p. 21° C., peak 19, dipentene, b.p. 178° C.) on a single injection of the sample. However, the infrared spectra of fairly broad peaks cannot be obtained because of the low concentrations in the carrier gas—e.g., spectra of the unnumbered peaks eluting between styrene and dipentene. Although the instrument yields a small spectral readout (21 × 17 cm.), suitable modification to improve this characteristic could be made easily.

Interestingly, several of the compounds identified in the ether codistillate have also been found in the methanol codistillate of cigarette (*1–3*) and cigar (*5*) smoke condensate. These compounds include toluene, ethylbenzene, *m*-xylene, *o*-xylene, styrene, and dipentene. Other aromatic hydrocarbons, 2,5-dimethylfuran, and a vinylcyclohexene reported in the methanol codistillate were not found in the present study. One hydrocarbon in the methanol codistillate, *p*-xylene, eluted with *m*-xylene under the chromatographic conditions used here and may, therefore, be present in the codistillate in amounts too small to detect. One of the components in the ether codistillate has not been previously reported in cigarette smoke: α-methylbutyraldehyde.

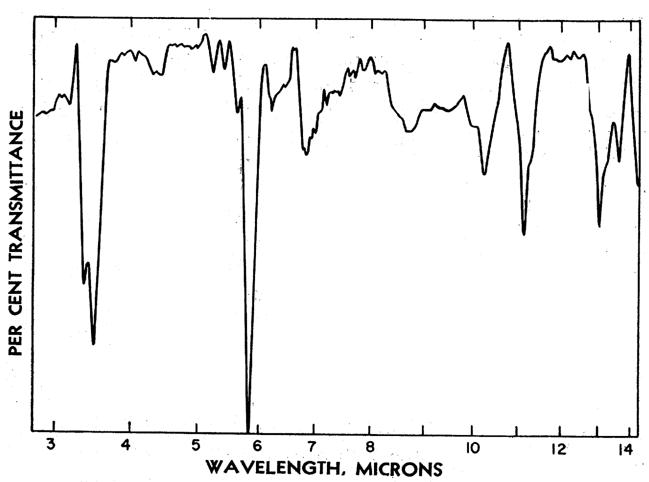

Figure 2. Infrared spectrum of peak 18 containing styrene and other compounds

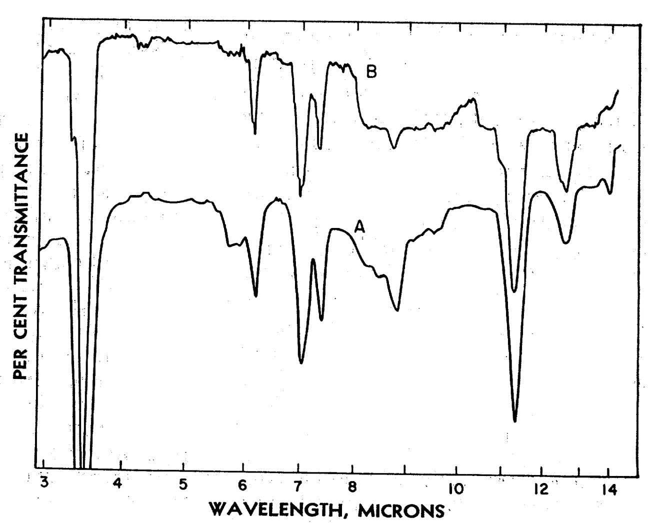

Figure 3. Infrared spectra of peak 19 from cigarette smoke fraction (*A*) and authentic dipetene (*B*)

Although infrared spectra were also obtained for peaks 2, 7 to 11, 14, and 15, the characteristics were not sufficiently distinct to claim tentative identification.

LITERATURE CITED

(1) Cook, J. W., Johnstone, R. A. W., Quan, P. M., *Israel J. Chem.* **1,** 356 (1963).

(2) Johnstone, R. A. W., Quan, P. M., *J. Chem. Soc.* **1963,** p. 2221.

(3) Johnstone, R. A. W., Quan, P. M., Carruthers, W., *Nature* **195,** 1267 (1962).

(4) Kelliher, J. M., Brown, R. A., *Abstracts*, p. 28, Eastern Analytical Symposium, New York City, November 1964.

(5) Osman, S., Barson, J., *Tobacco Sci.* **8,** 158 (1964).

(6) Stedman, R. L., Miller, R. L., *J. Chromatog.* **11,** 409 (1963).

(7) Wilks, P. A., Jr., Brown, R. A. ANAL. CHEM. **36,** 1896 (1964).

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